Ultraviolet and visible spectroscopy

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Lecture 5

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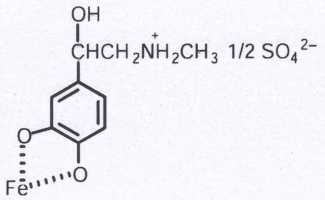
Fig. 4.13
The complex formed between adrenaline and iron, which is used to analyse adrenaline at low levels in an injection.



Adrenaline iron (II) complex

Self-test 4.7

Adrenaline in bupivacaine/adrenaline injection is assayed by complex formation with iron (II). 20 ml of the injection is mixed with 0.2 ml of reagent and 2 ml of buffer and a reading is taken in a 4 cm pathlength cell. A reading of a solution containing 5.21 μg/ml of adrenaline is taken under the same conditions. The following results were obtained:

- Absorbance of sample = 0.173
- Absorbance of standard solution = 0.181

Calculate the percentage of w/v of adrenaline in the injection.

Answer: 0.0005% w/v

Difference spectrophotometry

In difference spectroscopy, a component in a mixture is analysed by carrying out a reaction which is selective for the analyte. This could be simply bringing about a shift in wavelength through adjustment of the pH of the solution in which the analyte is dissolved or a chemical reaction such as oxidation or reduction. In the following example the selective alkaline shift of aspirin is used to determine it in a preparation also containing dextropropoxyphene, naphthalene sulphonic acid and caffeine. Caffeine, dextropropoxyphene and the naphthalene sulphonic acid anion do not undergo appreciable alkaline shifts whereas aspirin does. Figure 4.14A shows the spectrum of the extract from tablets in 0.1 M HCl – in fact there is relatively minor interference at the wavelength used for the determination of aspirin but by using the sample in HCl in place of a blank in the reference cell one can be sure that interference from the excipients is eliminated. Figure 4.14B shows the difference spectrum with the capsule extract in 0.1 M HCl in the reference cell and the capsule extract in 0.1 M NaOH in the sample cell. The absorbance at 299 nm is thus wholly due to aspirin. The problem remains of how to quantify the analyte in such a sample. This can be readily carried out using standard additions, which involves adding a known amount of aspirin standard to the sample and comparing the absorbance of the original sample extract with the absorbance of the spiked sample.

Analysis of aspirin in dextropropoxyphene compound tablets

Analysis was carried out by difference spectrophotometry. A one-point standard calibration for the determination of aspirin in dextropropoxyphene compound capsules was prepared by adding a known amount of aspirin to the sample from a

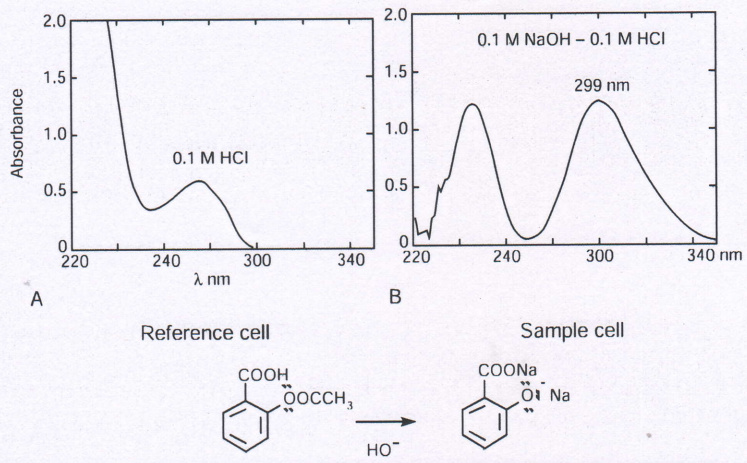


Fig. 4.14
UV difference spectrum used in the quantification of aspirin in dextropropoxyphene capsules.

standard stock solution. Stated content in the capsules: aspirin 250 mg, dextropropoxyphene napsylate 100 mg and caffeine 30 mg:

(i) 5 ml of the solution of sample in methanol is diluted to 500 ml with 0.1 M HCl: reference solution 1.
(ii) 5 ml of the solution of sample in methanol is diluted to 500 ml with 0.1 M NaOH.
(iii) 5 ml of sample solution and 5 ml of aspirin standard solution were mixed and diluted to 500 ml with 0.1 M HCl: reference solution 2.
(iv) 5 ml of the solution of sample in methanol and 5 ml of aspirin standard solution were mixed and then diluted to 500 ml with 0.1 NaOH.

Readings were taken at 299 nm of the sample solutions with and without standard addition against reference solutions 2 and 1, respectively. The following data were obtained:

- Weight of contents of 20 capsules = 10.556 g
- Weight of capsule content analysed = 0.1025 g
- Capsule contents were dissolved in methanol and adjusted to 100 ml
- Concentration of aspirin standard solution = 50.4 mg/100 ml
- Absorbance of sample at 299 nm in 0.1 M NaOH without standard addition = 0.488
- Absorbance of sample at 299 nm in 0.1 M NaOH with standard addition = 0.974.

Calculation example 4.5

In dilution (iii) aspirin standard solution is diluted 5 ml to 500 ml (× 100).

Concentration of aspirin standard in standard addition solution: $\frac{50.4}{100} = 0.504$ mg/100 ml.

The difference between the absorbance with standard addition and that without represents the absorbance due to a 0.504 mg/100 ml solution of aspirin.

Absorbance difference: 0.974 − 0.488 = 0.486.

(Continued)

Calculation example 4.5 *(Continued)*

Therefore, concentration of aspirin in the sample solution: $\frac{0.488}{0.486} \times 0.504 = 0.506$ mg/100 ml

Dilution factor for sample = 5 ml to 500 ml (× 100)

Concentration of aspirin in undiluted sample solution: $0.506 \times 100 = 50.6$ mg/100 ml

Volume of initial extract = 100 ml

Therefore, amount of aspirin extracted from the capsule powder = 50.6 mg:

Amount expected in capsule powder analysed: $250 \times 20 \times \frac{0.1025}{10.556} = 48.6$ mg.

Therefore, percentage of stated content: $\frac{50.6}{48.6} \times 100 = 104.1$.

Derivative spectra

Derivative spectra can be used to clarify absorption bands in more complex UV spectra. The technique is used extensively in the rapidly developing field of near-infrared spectrophotometry (see Ch. 5) and can also be applied in the determination of the purity of chromatographic peaks when they are monitored by diode array detection. The main effect of derivatisation is to remove underlying broad absorption bands where there is only a gradual change in slope. The first derivative spectrum is obtained by plotting, for instance, the slopes of 2 nm segments of the spectrum, and this results, as shown for a Gaussian band in Figure 4.15, in a spectrum where the slope is zero at the maximum of the peak and the slope is maximum at approximately half the peak height. In the second derivative spectrum the slopes of adjacent 2 nm segments are compared and this gives the points of maximum curvature of the spectrum. The rate of curvature of a spectrum has its greatest negative value at its maximum and the greatest rates of curvature are observed for narrow absorption bands. Figure 4.15 shows the first, second, third and fourth derivatives of a Gaussian band.

As would be expected, the first-order spectrum of pseudoephedrine, shown in Figure 4.16, gives maxima at the points where the slope is at a maximum in the zero-order spectrum. In addition, the second-order spectrum gives minima

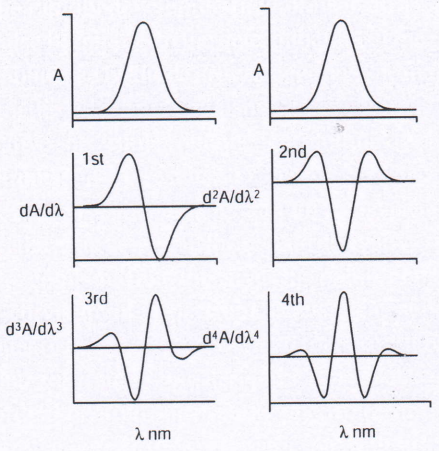


Fig. 4.15
Derivatives of a Gaussian absorption band.

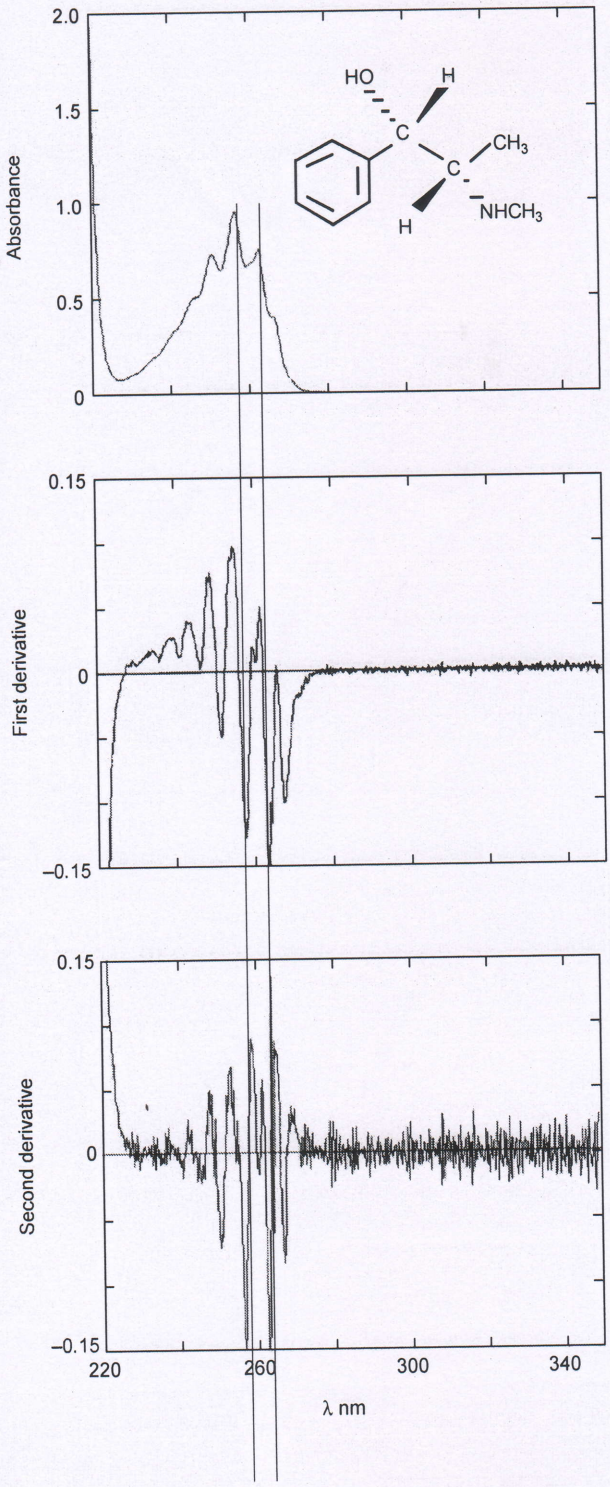


Fig. 4.16
UV spectrum of pseudoephedrine with its first and second derivative spectra. The minima correspond to the maxima in the absorbance spectrum.

The signal:noise ratio is poorer in the second derivative spectrum because, through dividing the spectrum into segments in order to calculate the derivative, the underlying noise is less efficiently averaged out, which occurs when the spectrum is scanned in much narrower segments.

Applications of UV/visible spectroscopy in preformulation and formulation

UV/visible spectrophotometry is a standard method for determining the physico-chemical properties of drug molecules prior to formulation and for measuring their release from formulations. The type of properties which can be usefully determined by the UV method are listed as follows:

Partition coefficient

The partition coefficient of a drug between water and an organic solvent may be determined by shaking the organic solvent and the water layer together and determining the amount of drug in either the aqueous or organic layer by UV spectrophotometry. If buffers of different pH values are used, the variation of partition coefficient with pH may be determined and this provides another means of determining the p*K*a value of a drug.

Solubility

The solubility of a drug in, for instance, water may be simply determined by shaking the excess of the drug in water or buffer until equilibrium is reached and then using UV spectrophotometry to determine the concentration of the drug that has gone into solution. Another method for determining solubility, where an ionisable group is present in the drug, is to dissolve varying concentrations of the salt of the drug in water and then add excess acid to a solution of the salt of an acidic drug or excess base to a solution of the salt of a basic drug, thus converting the drugs into their un-ionised forms. When the solubility of the un-ionised drug in water is exceeded, a cloudy solution will result and UV spectrophotometry can be used to determine its degree of turbidity by light scattering, which can be measured at almost any wavelength, e.g. 250 nm.

Release of a drug from a formulation

UV spectrophotometry is used routinely to monitor in vitro release of active ingredients from formulations. For simple formulations the drug is simply monitored at its λ max. In the example shown in Figure 4.18, the rate release of pseudoephedrine from a controlled release formulation was monitored.[1] The release of the drug was followed by monitoring its release into distilled water using a UV spectrophotometer set at 206 nm. In the example given in Figure 4.18, the particle size of the ethylcellulose used in the formulation affected the rate of release.

If UV-absorbing excipients were present in such a formulation, the UV wavelength used for monitoring release would need to be selected carefully, or HPLC coupled to UV detection might be used. For such studies the sampling of the dissolution medium may be fully automated so that the medium is filtered and pumped through to the UV spectrophotometer at set time intervals in order to take a reading.

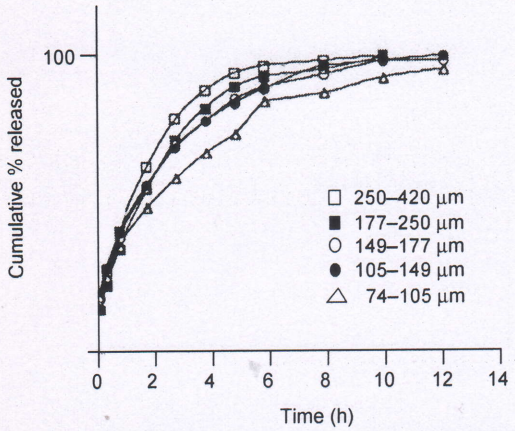


Fig. 4.18
Release of pseudoephedrine from a controlled release formulation. Reproduced with permission Kaitikaneni et al 1995 [1].

Additional problems

1. The BP assay for orciprenaline tablets is described below.
Weigh and powder 20 tablets. Shake a quantity of the powder containing 80 mg of orciprenaline sulphate with 50 ml of 0.01 M hydrochloric acid, filter and add sufficient 0.01 M hydrochloric acid to the filtrate to produce 100 ml. Dilute 10 ml to 100 ml with 0.01 M hydrochloric acid and measure the absorbance of the resulting solution at the maximum at 276 nm. Calculate the content of orciprenaline sulphate taking 72.3 as the value of *A* (1%, 1 cm) at 276 nm.

The following information was obtained during the assay:

Weight of 20 tablets = 2.5534 g

Weight of tablet powder assayed = 0.5266 g

Absorbance reading = 0.5878

Stated content per tablet = 20 mg.

Calculate the % of the stated content of the orciprenaline sulphate in the tablets.

Answer: 98.56%

2. Calculate the content of methoxamine hydrochloride in an injection from the following information:
The assay was carried out by diluting 1 ml of the injection to 100 ml with water and 20 ml of the diluted injection was then diluted to 100 ml. An absorbance reading of the final dilution was taken at 290 nm with a UV spectrophotometer:
Absorbance of diluted injection solution = 0.542
A 1% 1 cm value of methoxamine hydrochloride at 290 nm = 137.

Answer: 1.978% w/v.

3. Weigh and powder 20 tablets. Shake a quantity of the powder containing 20 mg of propranolol hydrochloride with 20 ml of water for 10 minutes. Add 50 ml of methanol, shake for a further 10 minutes add to produce 100 ml and filter. Dilute 10 ml of the filtrate to 50 ml with methanol and measure the absorbance of the resulting solution at the maximum at 290 nm. The assay of propranolol hydrochloride was carried out as described above. Using the following data calculate the % of stated content in the tablets:

Stated content per tablet = 10 mg

Weight of 20 tablets = 3.6351 g

Weight of powder taken for assay = 0.3967 g

A (1% 1 cm) at the maximum at 290 nm = 210

Absorbance reading obtained at 290 nm = 0.913.

Answer: 99.59%

(Continued)

Additional problems *(Continued)*

4. Weigh and powder 20 tablets. Shake a quantity of the powder containing 20 mg of warfarin sodium with 250 ml of 0.01 M sodium hydroxide for 15 minutes and filter. To 20 ml of the filtrate add 0.15 ml of hydrochloric acid and extract with three 15 ml quantities of chloroform. Extract the combined chloroform layers with three 20 ml quantities of 0.01 M sodium hydroxide. Dilute the combined aqueous layers to 100 ml with 0.01 M sodium hydroxide, filter and measure the absorbance of the resulting solution at the maximum at 307 nm. The assay of warfarin sodium was carried out as described above. From the data below calculate the % of stated content in the tablets:

Stated content per tablet = 3 mg

Weight of 20 tablets = 4.415 g

Weight of tablet powder assayed = 1.457 g

A (1%,1 cm) at the maximum at 307 nm = 431

Absorbance reading of diluted tablet extract at 307 nm = 0.6913.

Answer: 101.3%.

5. Shake a quantity of cream containing about 7.5 mg of acyclovir with 50 ml of 0.5 M sulphuric acid. Shake well with 50 ml of ethyl acetate, allow to separate and collect the lower aqueous layer. Wash the organic layer with 20 ml of 0.5 M sulphuric acid and dilute the combined washings and the aqueous layer to 100 ml with 0.5 M sulphuric acid. Mix well and filter. Discard the first few ml of filtrate and to 10 ml of the filtrate add water to produce 50 ml. Measure the absorbance of the resulting solution at the maximum at 255 nm. Using the data below calculate the %w/w of acyclovir in the cream:

Weight of cream analysed = 0.1564 g

Absorbance reading of diluted sample = 0.863

A (1%,1 cm) value at 255 nm = 562.

Answer: 4.91 %w/w.

References

1. Kaitikaneni PR, Upadrashta SM, Neau SN, Mitra AK. Ethylcellulose matrix controlled release tablets of a water-soluble drug. *Int J Pharm* 1995;**123**:119–25.

Further reading

Beckett AH, Stenlake JB. *Practical Pharmaceutical Chemistry, Part 2*. 4th ed. London: Athlone Press; 1988.

Clark BJ, Frost T, Russell MA. *Techniques in Visible and Ultraviolet Spectrometry*, vol. 4. London: Chapman and Hall; 1993.

Rojas FS, Ojeda CB. Recent development in derivative ultraviolet/visible absorption spectrophotometry: 2004–2008. *Analytica Chimica Acta* 2009;**635**:22–44.

Useful websites

www.spectroscopynow.com
The best web resource for spectroscopy. Contains useful links to tutorial materials and journals.

www.agilent.com
Many examples of applications of UV/visible spectroscopy and descriptions of instruments.

www.unicam.co.uk/com/cda/home
A wide range of instruments are made by this company.

www.anachem.umu.se/jumpstation.htm
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